Genes for Giants

t takes around 5 weeks for a tiny watermelon flower to grow into a ready-to-eat watermelon. Though ripe watermelons typically weigh anywhere from 5 to 40 pounds, record-breakers have tipped the scales at 250 pounds or more.

"Watermelon is a fruit that grows so fast, and it can get so big," says ARS plant geneticist Amnon Levi. "We know there must be some very interesting genes at work."

Levi works at the ARS U.S. Vegetable Laboratory in Charleston, South Carolina. He teamed up with plant pathologist Pat Wechter and plant geneticist Karen Harris on the first-ever study that identified and characterized key genes regulating watermelon growth and development.

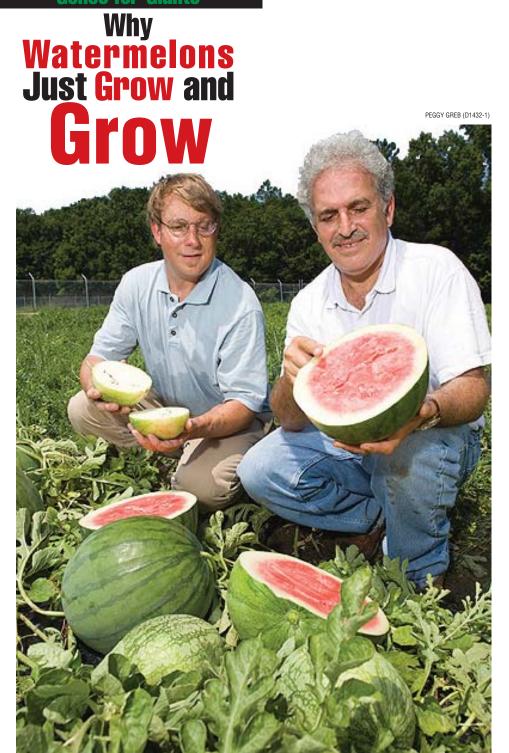
Over a 3-year period, plant geneticist Angela Davis, who works at the ARS South Central Agricultural Research Laboratory in Lane, Oklahoma, grew watermelons in the field for the research to ensure that the genes would be responding to actual field conditions, such as pathogens and weather extremes.

"One year it was so dry that we had to stop watering because water restrictions had been imposed," Davis notes. "The other 2 years it rained so much that the plants became stressed from too much water."

Davis extracted RNA from watermelon fruit at three stages of growth and ripening: at 12 days after pollination (DAP), when the flesh was white; at 24 DAP, when the flesh was pink; and at 36 DAP, when the flesh was red. Then she sent RNA from the tissue samples to Charleston for analysis.

Genes Prompt Plus-Size Fruit

Levi used this collection of RNA to develop a library of 832 expressed sequence tags (ESTs), which are unique gene segments that direct different aspects of development and metabolism. Then he worked with Wechter and Harris to decipher how the ESTs regulated plant growth and development.



Plant pathologist Pat Wechter (left) and plant geneticist Amnon Levi examine watermelon for fruit quality. They are studying gene expression in the three stages of watermelon ripening.

The team found that these ESTs were active in metabolism, cell growth, cell development, and transporting nutrients and other substances across cell walls. They also came into play in cell division, cellular communication, DNA copying,

plant defense, and stress response.

The Charleston researchers then identified significantly different levels of EST expression during the early, middle, and late stages of fruit growth and ripening. They found 335 ESTs that had at least

a twofold increase or decrease in copy number in at least one of the three stages. Of these 335 ESTs, 239 were very similar to ESTs found in other plants, while the remaining 96 had not been previously identified in any sequenced plant species.

"Most of these genes regulate targeted activities," Wechter says. "They are very specific genes that do certain things at a certain time during growth and ripening. Many of them are involved in the development of the watermelon vascular system, which is a crucial component for growth and ripening."

"The vascular system in a watermelon forms very fast because it is building the infrastructure for fruit, like building a highway before building a city," Levi says. "This system is how fluids transport sugars from the leaves into the fruit."

Ethylene Enters the Picture

But some of the results were unexpected—and may someday give breeders and producers an edge in moving watermelons to market.

In some types of fruit, ethylene gas is produced, and it is responsible for many ripening processes. These fruits are referred to as "climacteric fruits." In fact, producers often harvest climacteric fruits—such as tomatoes—before they are ripe, and then promote ripening by exposing the harvested fruit to ethylene.

But ethylene has not been linked to ripening of nonclimacteric fruits. Scientists consider watermelons and other cucurbits—along with grapes, citrus, and strawberries—to be nonclimacteric fruits. So Wechter and the others were very surprised to see differences in expression levels of genes involved in ethylene production in watermelon fruit. They then measured the amount of ethylene produced by developing and ripening fruit. They found a burst of ethylene production during the white-fruit stage and lesser amounts produced during the later stages.

"We just didn't think ethylene had any role in the ripening of watermelon,"

Wechter says. "Now we know it could be a central component of the ripening process. And if it's important in watermelon, it could be important in other nonclimacteric fruit as well."

Jim Giovannoni is a molecular biologist who works at the ARS Robert W. Holley Center for Agriculture and Health in Ithaca, New York. He maintains the Cucurbit Genomics Database (www.icugi. org), a gene bank for the Cucurbitaceae family that is used by researchers around the world. He helped the Charleston team sort through the watermelon ESTs.

"Finding ethylene activity in water-melon is significant because it shows the existence of the same type of genes that are also found in tomato and *Arabidopsis*—our model plant," Giovannoni says. "This shows that genes involved in model systems are also seen in crop systems, which validates the models we use. In addition, we have identified ESTs that could be used to develop molecular markers for ethylene response in watermelon."

Enigmatic ESTs Remain

Though the ESTs linked to ethylene activity were a surprise, the team was able to pinpoint their function. But the team also found 96 ESTs that remain somewhat of a mystery.

"They appear to be active all the time, which suggests they regulate more generalized, basic functions for plant survival," Wechter says. "But we can't match them up with any other known plant ESTs. We're trying to confirm they are unique to watermelon."

In 2008, U.S. watermelon crops valued at \$492 million were planted on just over 134,000 acres. But pathogens—including watermelon vine decline and *Phytophthora* blight—continue to threaten production. Finding sources of genetic resistance to these threats is essential to the success of watermelon crops across the southern United States.

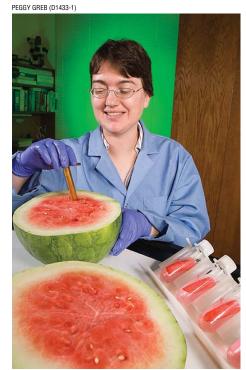
"Cultivated watermelons are not genetically diverse at all, which makes them much more vulnerable to pathogens and

environmental stresses," Harris notes. "It's difficult to find genetic differences in such a narrow selection of cultivars."

"I want to do more studies looking at the interaction of watermelon genes when the plant is challenged by pathogens," Wechter adds. "Our EST study is a good start. Now we need to build on this information and find ways to develop more resistant melons to meet the challenges in field production."—By **Ann Perry**, ARS.

This research is part of Plant Genetic Resources, Genomics, and Genetic Improvement (#301) and Plant Diseases (#303), two ARS national programs described on the World Wide Web at www.nps.ars.usda.gov.

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Plant geneticist Karen Harris removes watermelon flesh for RNA extraction.